Introduction to Next Generation Sequencing Technology for Non Practitioners

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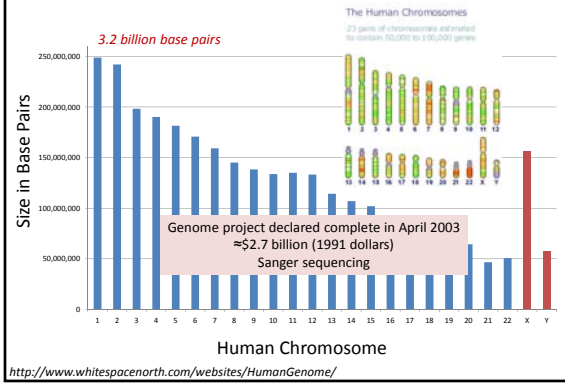
Global Identity Summit
DNA: Next Generation Sequencing Technology Application to Human ID
September 22, 2015

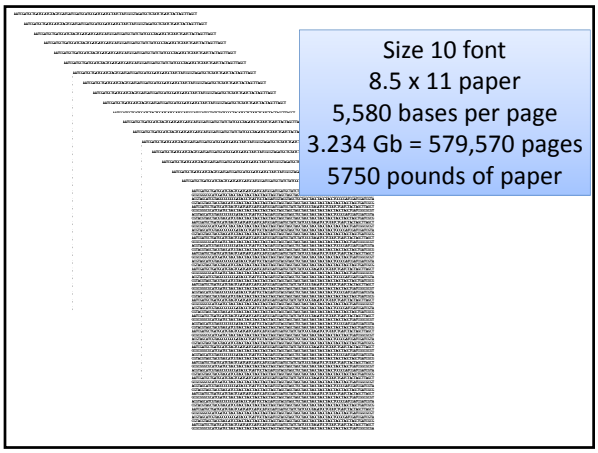
Outline

- Human Genome
- Sanger sequencing
- 'Disruptive innovation'
- Non-identity applications
- Generalized workflows for NGS
- Platforms and throughput
- Sequencing chemistries
- Wrap up / thoughts

Human Genome



http://www.whitespacenorth.com/websites/HumanGenome/



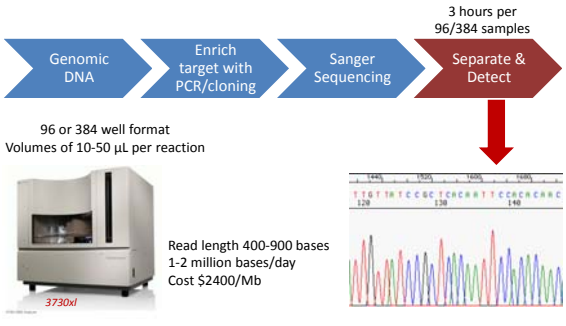
Sanger Sequencing

How the human genome was first completed April 2003

- 3.2 billion bp
  - Each chromosome 50 to 300 million bp
  - Clone 150-200 million bp pieces into bacteria (BAC)
  - Store fragments and replicate
  - 20,000 BACs for the human genome
  - Each BAC is cut into 2,000 bp
  - Sequenced 500-800 bp (10x) depth
  - Map to chromosome and assemble…

Generalized Sanger Workflow

Fluorescently labeled DNA fragments are separated and detected by capillary electrophoresis



What's in a name???

Massively parallel sequencing

NGS

Second-generation sequencing

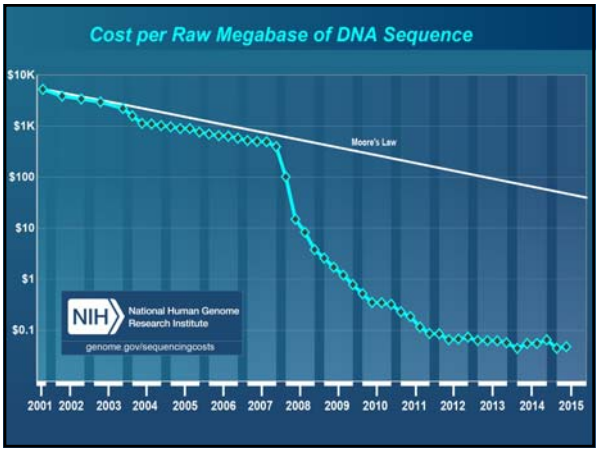
Next-generation sequencing

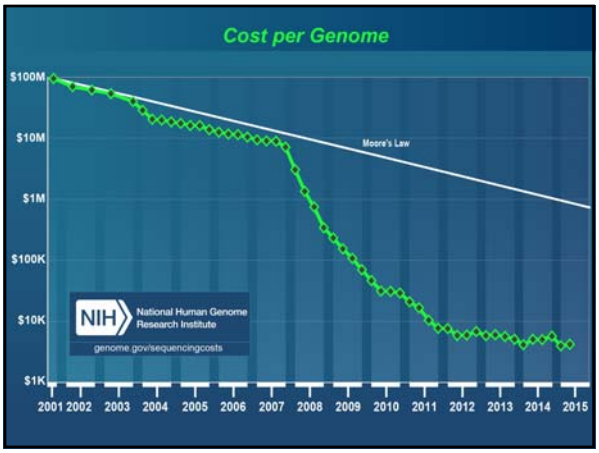
Whole-genome sequencing

Third-generation sequencing

HIGH-THROUGHPUT SEQUENCING

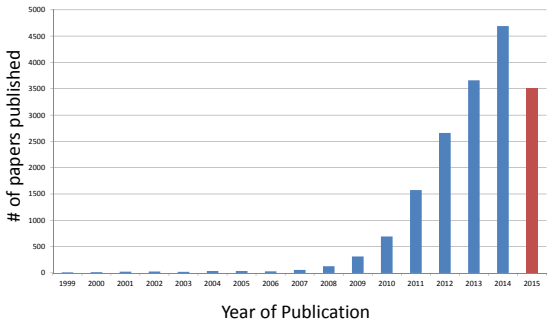
Next-generation genomics





PubMed Search results for "Next Generation Sequencing"

http://www.ncbi.nlm.nih.gov/pubmed



Non-identity applications

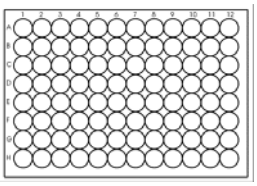
- Clinical
- Inherited disease
- Reproductive health
- Cancer – gene fusion
- Rare variants
- Pre-implantation (genetic screening)
- Transplant medicine (HLA)
- Microbiomics/Metagenomics
- Gene expression | RNA seq
- Public health
- Ancient DNA
- NIPT (non-invasive prenatal testing)

Next Generation Sequencing

Massively Parallel Sequencing

'Millions of wells'

- One DNA fragment per 'well'
- Typically, shorter reads (Range 75 to 400)
- High coverage 100 – 1000 - 10,000x
- **Rely more on informatics to assemble millions of short reads**

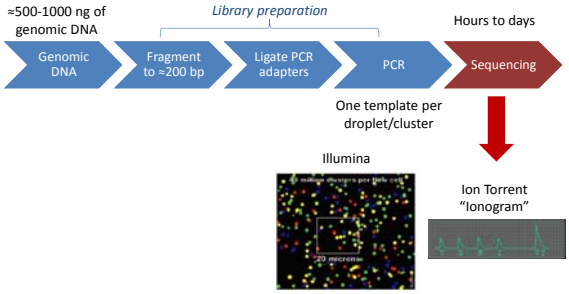


- Each well of the plate will generate 500-900 bp of sequence data
- One plate = 86,400 bp of sequence data
- 3 h per run (max 8 runs/day)
- 691,200 (≈ 0.7 mb per day)

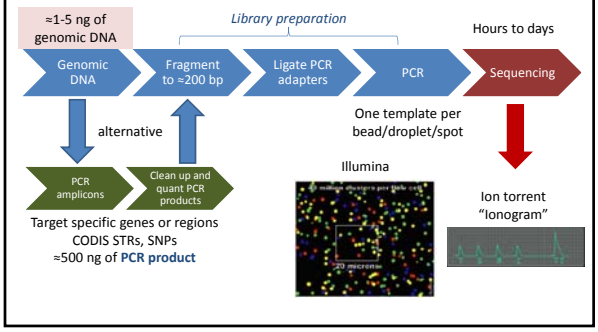
- **Millions of 'wells' generating 200-400 bp of sequence data**
- **nL of reagents per reaction**
- **2 hours to 6 days per run**
- **0.5 - 1800 Gb of sequence data per run**

Massively parallel sequencing takes advantage of partitioning of molecules

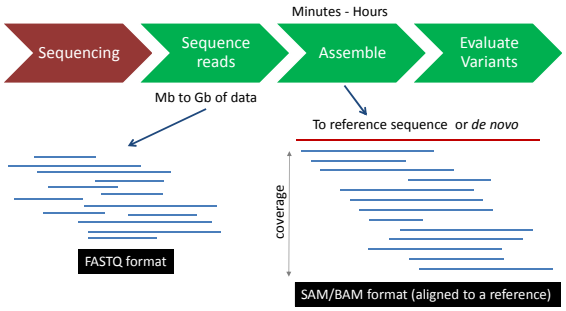
Generalized NGS Workflow



Generalized NGS Workflow



Generalized NGS Workflow



FASTQ Format

- FASTQ - normally uses four lines per sequence.
- Line 1 begins with a '@' character and is followed by a sequence identifier and an *optional* description
- Line 2 is the raw sequence letters.
- Line 3 begins with a '+' character and is *optionally* followed by the same sequence identifier (and any description) again.
- Line 4 encodes the quality values for the sequence in Line 2, and must contain the same number of symbols as letters in the sequence.
- (1)@SEQ_ID
  (2)GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCC
  (3)+
  (4)!''*((((***+))%%%++)(%%%%).1***-+*''))**

http://maq.sourceforge.net/fastq.shtml

Informatics

After the generation of sequence data

- Storage of data
- Assembly and parsing of data
- Variant calling
- Applied use of information
- Hire a bioinformatics expert…

Sampling of NGS Platforms

- Illumina
  - **MiSeq**
  - **FGx**
  - HiSeq 2000/2500
  - NextSeq
- Life Technologies
  - SOLiD (5500 series)
  - **Ion Torrent PGM**
  - Ion Torrent Proton
- Pacific Biosciences
  - PACBIO RS II
- Oxford Nanopore
  - MinION
- 454 Roche
  - GS jr
  - GS FLX+





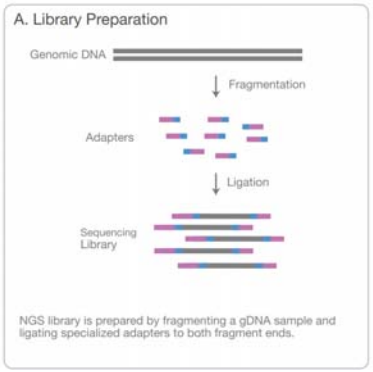
October 15, 2013 – Roche shutting down 454 sequencing business
Will be phased out mid-2016

NGS Chemistry

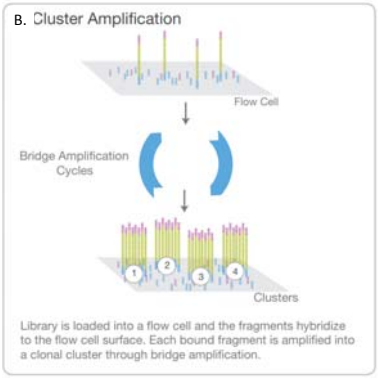
- Not Sanger sequencing
- Examples
  - Sequencing by synthesis (Illumina)
  - Ion semiconductor sequencing (Ion Torrent)
  - Single molecule real time (Pacific Bioscience)
  - Nanopore sequencing (Oxford Nanopore)

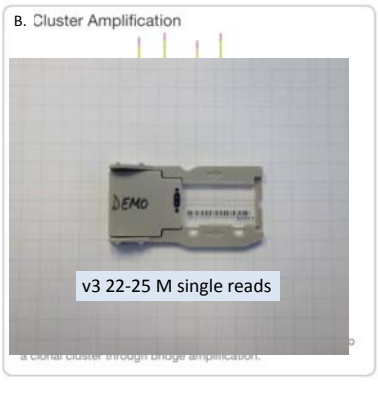
Illumina MiSeq

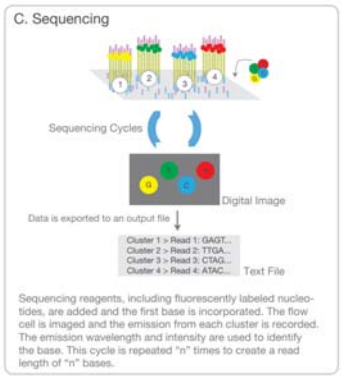
- MiSeq launched in Jan. 2011
- The MiSeq uses a sequencing by synthesis approach:
  - Nextera enzymatically fragments and tags DNA
  - Limited cycle PCR
  - Flow cell hybridization
  - Bridge PCR - clusters
- Fluorescent light detection
  - Each base has a unique color
  - Sequence each end of the molecule



http://www.illumina.com/content/dam/illumina-marketing/documents/products/illumina_sequencing_introduction.pdf



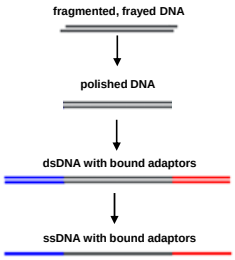




Life Tech - Ion Torrent - PGM

- Ion Torrent launched in Feb. 2010
- Ion Torrent sequencing employs an analogous technique as pyrosequencing:
  - Emulsion PCR for single copy reactors
  - Non-labeled nucleotide triphosphates are flowed over a bead on a semiconductor surface
- Hydrogen Ion detection
  - pH change is detected
  - **No optics**

Constructing a Library

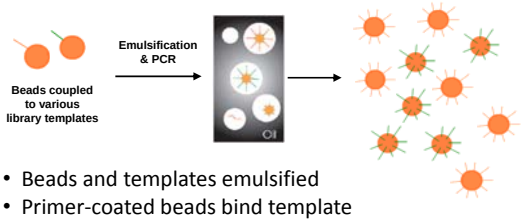


- Frayed ends are enzymatically "polished"
- Adaptor oligos ligated onto fragments
- Denatured to ssDNA
- Emulsified with primer-coated beads
- Hybridization of template to bead

Margulies et al. (2005) Supplementary Materials

Ion Torrent - PGM

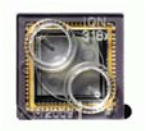
Emulsion PCR & Enrichment



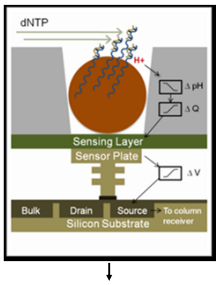
- Beads and templates emulsified
- Primer-coated beads bind template
- PCR amplifies template
- Enrich for beads containing PCR products
  - magnetic capture
- Adaptable to automation (Ion Chef)

Dressman et al. (2003)

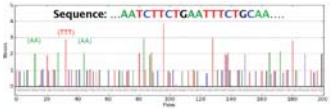
Ion torrent PGM chip



'318' chip
11 M sensors



- Chip flooded with one nucleotide after another
- H^+ released when a complementary base is added to template
- Charge from the ion causes detectable pH change
- Sequencer calls the base



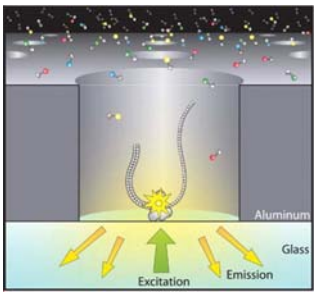
Life Technologies

Ion Torrent - PGM

Pacific Biosciences

- Instrument = PacBio RS II (2011)
- SMRT = single molecule real time
- NTPs are attached to an unique fluorescent dye
  - **A**, **C**, **G**, **T**
- Sequence is 'read' as the template is being synthesized (in real time)
- Long reads >14,000 bp

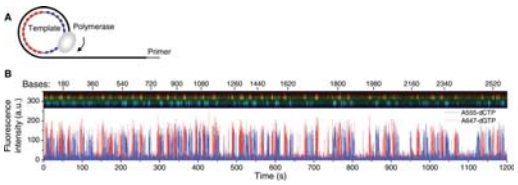
SMRT = single molecule real time



- DNA polymerase is immobilized at the bottom of the well
- Fl-labeled ACGT diffuse into the well and are incorporated
- Light is detected as dNTPs are incorporated

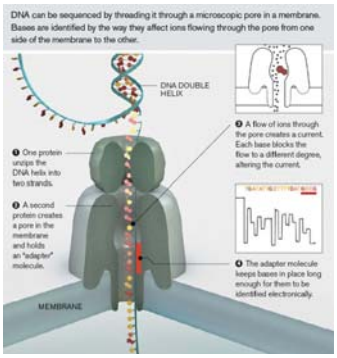
http://chrisamiller.com/science/2010/02/10/third-gen-sequencing-pacific-biosciences/
Eid, et al. Real-Time DNA Sequencing from Single Polymerase Molecules. Science doi:10.1126/science.1162986

SMRT = single molecule real time



http://www.sciencemag.org/content/323/5910/133/F3.large.jpg

Nanopore Sequencing



http://www2.technologyreview.com/article/427677/nanopore-sequencing/

Nanopore Sequencing

http://www2.technologyreview.com/article/427677/nanopore-sequencing/

NGS Specs as of Sept 2015

	NGS Platform	Capacity/Output	Read Length	Approx run time
ThermoFisher	Ion PGM	30 Mb to 2 Gb (1.2-11 M)	200-400 bp	2 hours
	Ion Proton	Up to 10 Gb (165-660 M)	200 bp	2-4 hours
Illumina	MiSeq Series	15 Gb (25 M)	2 x 300 bp	5-65 h
	HiSeq Series	1500 Gb (5000 M)	2 x 150 bp	40 h to 6 days
	NextSeq Series	120 Gb (400 M)	2 x 150 bp	12-30 h
	HiSeq X Series	1800 Gb (6000 M)	2 x 150 bp	3 days
Pacific Biosciences	PACBIO RS II	Up to 1 Gb (150 k)	> 20 kb	0.5 to 4 h

[1] https://www.illumina.com/content/dam/illumina-marketing/documents/products/brochures/brochure_sequencing_systems_portfolio.pdf
[2] http://files.pacb.com/pdf/PacBio_RS_II_Brochure.pdf
[3] https://www.thermofisher.com/us/en/home/life-science/sequencing/next-generation-sequencing/ion-torrent-next-generation-sequencing-workflow/ion-torrent-next-generation-sequencing-run-sequence/ion-proton-system-for-next-generation-sequencing/ion-proton-system-specifications.html

Moving Targets

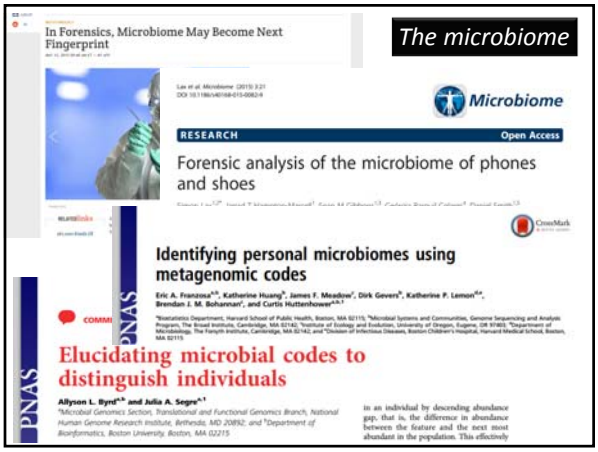
6 months from now these parameters will have changed

- Newer instruments
- Costs decreasing
- Throughput increasing
- Read lengths increasing
- Chemistries improving
- Library preparations – simpler/automated
- Computers faster – data storage cheaper
- Platforms leaving the market (e.g. Roche 454)
- Platforms entering the market (e.g. Qiagen GeneReader, Oxford Nanopore MinION)

Use of NGS for identity applications

Highly-parallel/high-throughput direct sequencing of relevant targets

- Forensic and/or biometric genetic markers
  - newer human identity applications
  - biogeographical ancestry, externally visible traits, complex kinship
  - degraded samples, mixtures, low template
- Traditional Forensic Markers
  - Whole mitochondrial genome analysis
  - Going in depth into STR loci and beyond



General Comments

- NGS is a 'disruptive innovation'
- NGS platforms/technologies open up high throughput access to genomic information
  - Whole genome or targeted regions
- The amount of sequence data generated makes the technique economical
  - Information per unit time
  - Current cost: days and thousand of dollars per run
  - BUT always decreasing and automation is helping
- Bioethics?

Not rapid/'field ready'

Thanks for your attention!

Questions and discussion?

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301-975-4872

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Outside funding agencies:
FBI - Evaluation of Forensic DNA Typing as a Biometric Tool